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13. ABSTRACT *(Maximum 200 Words)*
Most research that requires the long-term propagation of prostate cancer (PCA) cells in culture is carried out with three cell lines: DU 145, PC-3, and LNCaP. All of these lines were derived from metastases; only one, LNCaP, makes prostate specific antigen (PSA) and/or androgen receptor. Neither DU 145 nor PC-3 exhibit any phenotypic markers specific for prostatic epithelial cells. Better models are needed. There is evidence that some prostatic fluids contain PCA cells. For this proposal, our goals are (1) to test the tumorigenicity of and to develop transplantable xenografts from PCA cells in prostatic fluid, (2) to develop methods for enhancing the tumorigenicity of small numbers of these PCA cells without deliberately altering their genes, (3) to test these methods for enhancement of tumorigenicity with prostatic fluid cells, and (4) to initiate clinical follow-up. To date, we have detected significant, often sustained elevations of PSA in the blood of several mice that received prostatic fluid cells for many months after injection. In the case of two mice that received prostatic fluid cells from one patient, PSA increased progressively over the course of a year but started to fall at the 12-month time point. Cells from the injections sites were transplanted; one recipient has an elevated PSA. We have studied the coinjection of lethally irradiated, growth-factor-producing cells from several tumors with encouraging results in the case of some tumors.

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Introduction

The development of new approaches to the treatment of prostate cancer (PCA) would be greatly facilitated if there were a larger number of experimental models available. As we reviewed two years ago (Pretlow and Pretlow, 2000), (a) the rodent prostate seems almost irrelevant to us since it is biochemically, functionally, and embryologically quite different from the human prostate and (b) there are very limited numbers of human PCA epithelial cells that can be grown in the laboratory. Tissue culture lines derived from human PCAs are successful in less than 0.1% of human PCAs tested, and most such lines fail to make two molecules that are very important for most human PCAs: androgen receptor and prostate specific antigen (PSA). There are laboratories, including our own, that have reported the development of PCA xenografts from 5-10% of human PCAs; and most such xenografts make both PSA and androgen receptors, i.e., have functional properties in common with most human PCAs. If an investigator wants to develop a human PCA tissue culture line from a xenograft, there is an infinite supply of xenograft available, and tissue culture lines have been established from xenografts. One such line was developed from one of our xenografts (Sramkoski et al., 1999) and is available from the American Type Culture Collection (their catalogue number CRL-2505). Our goals in the proposed research are to assess the tumorigenicity of PCA cells in prostatic fluid, to develop methods to enhance the tumorigenicity of the small number of cells available there, to test those methods for the enhancement of tumorigenicity of small numbers of PCA cells, and to initiate clinical follow-up to find any correlations that may exist between the clinical courses of patients and whether or not their cells grow to form xenografts.

Body

Specific Aim 1 (also task 1). To test the tumorigenicity of cells obtained from the prostatic fluids (PF) of patients with PCA and to develop serially transplantable xenografts from these cells.

In months 1-18, we (statement of work, task 1 of the proposal) proposed to test the tumorigenicity of prostatic fluid cells obtained by prostatic massage from 30 patients. Our testing was delayed initially because our institution renovated some of the nude mouse facility and did not finish that task until approximately four months after this grant started. This restricted severely the number of cages available to our laboratory for several months.

In our first year, as described in our annual report a year ago, we injected cells from the prostatic fluids of eleven patients into nude mice. Blood has been drawn from these mice 1, 2, 4, and 6 months (as the mice reached those intervals) after the injection of the cells. Elevated blood PSA has been observed in six mice injected with prostatic fluid cells from five patients; however, the elevated PSA had been of low magnitude (0.02-0.10 ng/ml). To date, we have not observed tumor histologically at the site of injection or in the lungs of mice injected with PF cells.

Among the mice being followed from this group that was injected during this first year of this project, two animals (that received prostatic fluid from a single patient) became very important during the second year of the project. These mice, mouse numbers 10028 and 10030, developed elevated blood levels of PSA that progressed from 0.14 and 0.03 ng/ml four months after receiving the patient's cells to 1.67 and 2.13 ng/ml ten months after injection. The 12-month time point was the first time interval after the levels became detectable when the PSA levels fell. They fell significantly to from 1.67 to 1.24 and from 2.13 to 1.45 ng/ml, respectively. Two weeks later, the change in course of PSA over time was confirmed with levels of 1.2 and 1.14 ng/ml. The animals were killed one-half week and one week after these determinations, at which time the PSA values were 1.06 and 1.11 ng/ml. The sites of injection were obtained as suspensions of viable cells as described previously and injected into

additional mice. One of the recipients of these cells has shown an abnormal PSA level (0.06 ng/ml) at four months after injection; all recipients of these cells will have their peripheral blood assessed for PSA in two more weeks. The sustained, progressive increase in PSA over ten months and the elevation of PSA in the mouse that received the second generation of cells from this patient is strong evidence that human prostatic fluid cells do contain prostate cancer cells that can grow as xenografts. The fundamental problem that we are addressing is: how can we make human prostate cancer cells grow faster so as to form transplantable tumors in nude mice? The solution of this problem is critical and constitutes the goal of Specific Aim 2 (also task 2).

Among the mice that received prostatic fluid from the eleven patients whose cells were injected into mice in the first year of this project, mice that received cells from six patients developed detectable levels of PSA. Except for the patient described above, the cells from other patients failed to give sustained PSA levels beyond six months. The highest levels observed was 0.18 and 0.23 ng/ml in the mice that received cells from one patient at the four-month time point; however, these levels of PSA had returned to being undetectable at six months.

In the second year of this project, mice have received cells from the prostatic fluid of eleven additional patients. Mice that received cells form eight of these patients have exhibited abnormal PSA levels. The most elevated of these are PSA levels in two mice that received cells from a single patient; there PSAs are 0.16 and 0.19 ng/ml at six months. These animals will be monitored at intervals of two months. Those whose PSA becomes normal will be killed. Those whose PSA reaches higher levels will be used for transplantation of cells obtained in suspension from the site of injection.

Specific Aim 2 (also task 2). To develop methods for enhancing the tumorigenicity of small numbers of PCA cells without deliberately altering their genes.

As noted in our annual review a year ago, specific Aim 2 is particularly important since the successful conduct of the proposed work for specific aim 3 is dependent on the identification of tumor cells that can be irradiated and coinjected with CWR22 with enhancement of the rate of growth of CWR22. Success in specific aim 2 might also have enormous significance for areas that are not directly related to this research proposal. For example, there are those who are interested in growing PCA cells from the blood of patients with PCA. We reported the growth as xenografts of PCA cells from the blood of two of eleven patients with metastatic PCA (Pretlow et al., 2000); however, if this is to provide a useful means of sampling patients' metastatic tumor for the purpose of predicting the appropriate drugs for specific patients, any means that would permit the growth of PCA xenografts from a higher proportion of PCA patients would greatly facilitate this kind of research.

As noted a year ago, in the first year of this project, we tested the coinjection of 250 CWR22 prostate cancer xenograft cells, a marginally tumorigenic dose, with graduated doses of lethally irradiated cells intended as cells that might make paracrine growth factors that might accelerate the growth of CWR22.

As detailed in the annual report a year ago, by the end of the first year, we had tested DU 145 and CWR91 as lethally irradiated, coinjected feeder cells. As noted, we did a final experiment with DU 145 coinjected cells in the second year of this project for which this is the annual report. DU 145 cells were without significant effect; CWR91 cells appeared to suppress the growth of CWR22, probably because of the production of TGF-beta.

In the second year of this project, we have tested the coinjection of 250 CWR22 prostate cancer xenograft cells with a wide range of doses (approximately 100,000 to 20,000,000) of xenograft cells with the goal of determining (a) whether of not specific xenograft cells could

enhance the growth of CWR22 and (b) what the optimal dose for coinjection might be. The xenograft lines tested during the past year were purchased from the Biological Testing Branch of the National Cancer Institute. We were unable to propagate two of the lines received from this source: BT-549 and JS 578T. The lines tested were LOX IMVI (one experiment), SNB-75 (four experiments), SF-295 (four experiments), NCI-H23 (two experiments), MDA-MB-231/ATCC (one experiment), and K562 (one experiment). For these experiments, in addition the animals that received the graduated doses of coinjected xenograft cells, 5 control animals per experiment received 250 CWR prostate cancer xenograft cells without any coinjected, lethally irradiated cells from another xenograft line.

In the first experiment with a xenograft line, when the tumor sites that received coinjected xenograft cells mixed with CWR22 cells grew less well than the sites that received only CWR22 cells, the coinjected, feeder line was eliminated as a prospective choice; and the propagation of the line in our laboratory was terminated. The lines that were terminated in the second year of this project after the first experiment were KG-1A, LOX IMVI, and MDA-MB-231/ATCC. Another line that was very promising but not as consistent in behavior as we would like was SNB-75. Some experiments with that line were very promising; others were not. After four experiments, that line was terminated.

In the second year of this project, the lines that have been tested at least once and which are sufficiently promising to merit continued propagation for further testing are K562, NCI-H23, and SF-295. SF-295 has given very promising results. In an experiment with SF-295 that is still in progress after eight months, one in five animals that received 250 CWR22 cells in the absence of coinjected SF-295 cells developed a tumor is less than one hundred days. In contrast, six of ten animals that received coinjected SF-295 developed tumor in less than one hundred days.

Specific aim 3 (also task 3) is dependent upon success in specific aim 2. Progress on specific aim 2 is proceeding rapidly, and we shall start specific aim 3 after tumor lines are identified that markedly enhance the growth of CWR22 when irradiated and coinjected.

Specific aim 4 (also task 4) is being pursued with the collection of the clinical data described in that aim for use in future correlations between clinical data and the extent of our success in growing patients' prostatic fluid PCA cells as xenografts.

Key Research Accomplishments

As was the case in the first year of this project, prostatic fluid cells from eleven patients have been injected into nude mice, i.e., a total of twenty-two patients for the first two years. As detailed above, the cells from one of these patients gave increasing PSA levels in the blood of the mouse for ten months. To date, no other laboratory has observed this phenomenon. These experiments are being continued with observation of the recipients of prostatic fluid cells until tumor develops or PSA returns to undetectable.

Testing of KG-1A, LOX IMVI, MDA-MB-231/ATCC, and SNB-75 as irradiated feeder cells to be coinjected with PCA cells in completed.

Testing of K562, and NCI-H23 and SF-295 as irradiated feeder cells to be coinjected with PCA cells has been started.

Reportable Outcomes

There are no reportable outcomes.

Conclusions

To date, the most significant conclusion is that prostatic fluid contains prostate cancer cells that can grow in nude mice and cause elevation of prostate specific antigen (PSA) in the blood of mice. The cells grow slowly in nude mice and have not grown sufficiently rapidly to cause tumors greater than 3 mm in diameter at the sites of injection. Successful propagation of human prostate cancers derived from prostatic fluid cells will require continued testing of lethally irradiated cells that produce growth factors capable of accelerating the growth of human prostate cancer cells.

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Appendices

No appendices are included.